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Sulodexide prevents peripheral nerve damage in streptozotocin induced diabetic rats

Heung Yong Jin [a], Kyung Ae Lee [a], Sun Kyung Song [a], Wei Jing Liu [a], Ji Hae Choi [a], Chang Ho Song [b], Hong Sun Baek [a], Tae Sun Park [a,*]

[a] Division of Endocrinology and Metabolism, Department of Internal Medicine, Research Institute of Clinical Medicine, Chonbuk National University Medical School, Jeonju, South Korea
[b] Department of Anatomy, Chonbuk National University Medical School, Jeonju, South Korea

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ABSTRACT

We investigated whether sulodexide has additional protective effects against peripheral nerve damage caused by microvascular dysfunction in a rat model of diabetes. Female Sprague–Dawley (SD) rats were divided into the following 4 groups (n = 7–9/group): Normal, Normal + Sulodexide (sulodexide 10 mg/kg), diabetic group, and diabetic + Sulodexide (sulodexide 10 mg/kg). We assessed current perception threshold, skin blood flow, superoxide dismutase, and proteinuria in experimental rats after oral administration of sulodexide for 20 weeks. We also performed morphometric analysis of sciatic nerves and intraepidermal nerve fibers of the foot. Superoxide dismutase activity in the blood and sciatic nerve were increased significantly after sulodexide treatment in the diabetic group. Current perception threshold was reduced at 2000 Hz (633.3 ± 24.15 vs 741.2 ± 23.5 μA, $P<0.05$) and skin blood flow was improved (10.90 ± 0.67 vs 8.85 ± 0.49 TPU, $P<0.05$) in the diabetic + Sulodexide group compared with the diabetic group. The mean myelinated axon area was significantly larger (56.6 ± 2.2 vs 49.8 ± 2.7 μm^2, $P<0.05$) and the intraepidermal nerve fiber density was significantly less reduced (6.27 ± 0.24 vs 5.40 ± 0.25/mm, $P<0.05$) in the diabetic + Sulodexide group compared to the diabetic group. Our results demonstrate that sulodexide exhibits protective effects against peripheral nerve damage in a rat experimental model of diabetes. Therefore, these findings suggest that sulodexide is a potential new therapeutic agent for diabetic peripheral neuropathy.

1. Introduction

Peripheral neuropathy is the most common and debilitating complication of diabetes; thus the pathophysiology and therapeutic development of diabetic peripheral neuropathy are active areas of research. Diabetic peripheral neuropathy is thought to occur as a result of hyperglycemia-induced damage to nerve cells and from neuronal ischemia caused by hyperglycemia-induced decreases in neurovascular flow (Edwards et al., 2008; Vinik and Mehrabyan, 2004). Hyperglycemia causes systemic oxidative stress and increases inflammation through cytokine secretion by various cells (Aronson, 2008). Therefore, new agents that improve vascular blood flow by endothelial protection or increasing anti-oxidative stresses have been tested as novel treatments for diabetic peripheral neuropathy.

Sulodexide is a mixture of glycosaminoglycans that includes low molecular weight heparin and dermatan sulfate, both of which have antithrombotic and profibrinolytic properties (Ofosu, 1998). Sulodexide also exhibits endothelium-protective (Kristova et al., 2008) and anti-inflammatory effects in diabetes. Previous studies have shown that glycosaminoglycans and proteoglycans exert anti-inflammatory activity in various cells (Neumann et al., 1999; Wang et al., 2006) and high molecular weight hyaluronic acid down-regulates the gene expression of osteoarthritis-associated cytokines and enzymes in fibroblast-like synoviocytes from patients with early osteoarthritis (Ciszewicz et al., 2009). The clinical efficacy and safety of sulodexide have also been demonstrated in peripheral arterial disease, cardio-cerebrovascular disease, nephropathy, and postphlebitic syndrome (Gambaro et al., 1992; Harenberg, 1998; Williams, 2006).

Potential therapeutic applications of sulodexide for the treatment of diabetic peripheral neuropathy are based on the pathogenesis of diabetic peripheral neuropathy and the diverse effects of sulodexide. The purpose of this study was to demonstrate the protective effects of sulodexide on peripheral nerves in a rat model of experimental diabetes.

2. Materials and methods

2.1. Rats and induction of diabetes

Female Sprague–Dawley (SD) rats (160–180 g, 6–8 weeks old) were purchased from Damool Science (Daejeon, Chungnam, Korea) and allowed to adapt to their new environment for 1 week. Rats were kept in a pathogen-free rat-rearing facility with a 12 h light and dark

* Corresponding author at: Division of Endocrinology and Metabolism, Department of Internal Medicine, Chonbuk National University Medical School, 634-18, Keum-Am Dong, Jeonju, 561-712, South Korea. Tel.: +82 63 250 1794; fax: +82 63 254 1609.
E-mail address: pts@chonbuk.ac.kr (T.S. Park).

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cycle. The temperature (23 ± 1 °C) and humidity (53 ± 2%) of the room were strictly maintained and rats were provided with food and water *ad libitum*. Single intraperitoneal injections of streptozotocin (60 mg/kg body weight) (Sigma Chemical, St. Louis, MO, USA), dissolved in 0.1 mol/l citrate buffer (pH 4.5), was used to induce diabetes. Age-matched rats used as normal glucose controls received an equal volume of vehicle (sodium citrate buffer) administered in the same manner. Forty-eight hours after the injection of streptozotocin, rats with blood glucose levels higher than 350 mg/dl after overnight fasting were considered to be diabetic. The Precision Xtra Plus® (Abbot Laboratories, MediSence Products, Bedford, MA, USA) system was used to measure glucose.

2.2. Intervention schedule

At least 2 weeks after the injection of streptozotocin was needed to induce the typical features of diabetes. Four weeks after the verification of diabetes, normal and diabetic rats were randomly assigned to the following 4 groups (n = 7–9/group): Normal, Normal with sulodexide, diabetic group, and diabetic with sulodexide. Sulodexide (supplied by Asia Pharm. Korea) was dissolved in water and administered orally at a dose of 10 mg/kg. Sulodexide doses were tested from 0.125–0.5 LRU/ml (Ciszewicz et al., 2009) or 2–10 mg/kg for anti-thrombotic effect without bleeding problems (Harenberg, 1998) in previous animal studies and usual doses are 500 LRU/day or 100 mg/day in humans. Therefore, a 10 mg/kg dose was selected in this study to determine the neuroprotective effect. Normal and diabetic rats were treated with compound or placebo once a day for 20 weeks. The protocol for rat care and experimental procedures were approved by the Institutional Rat Care and Use Committee of the Chonbuk National University Medical School.

2.3. Body weight, blood glucose, HbA1c, and urinary protein measurements

Body weight and blood glucose were measured every week after 8 h of fasting. Fasting glucose levels were assessed using blood samples drawn from a tail vein and HbA1c levels were compared using a commercially available kit (NycoCard, Oslo, Norway). On the second day of the 20th week, a glucose tolerance test was performed by administering 50% dextrose (2 g dextrose/kg) into the stomach of the rats after overnight fasting. Before and after administration (0, 0.5, 1, 2, and 3 h), blood glucose levels were also assayed. Plasma was collected after centrifugation for 10 min at 1000 × *g* at 4 °C. The 24 h urine of each rat was collected with a metabolic cage and the 24 h protein excretion and urinary albumin(mg/dl)/creatinine(g/dl) ratio (ACR) levels were determined.

2.4. Current perception threshold

Current perception thresholds were examined to quantify nerve dysfunction during the 20th week, as in our previous study (Jin et al., 2009b). In brief, 2000, 250 and 5 Hz frequency stimuli were used to determine the perception thresholds with respect to pressure, vibration, and temperature, respectively. The sine-waves were delivered by a Neurometer® CPT/C (Neurotron, Inc., Baltimore, MD, USA) and the intensity of the 2000, 250 and 5 Hz stimuli were increased to 0.04, 0.02 and 0.01 mA/s, respectively. The current perception threshold was defined as the minimum intensity value required to elicit a withdrawal reflex of the hind paw or appearance of vocalization or agitation. When the response occurred, the stimulus was immediately stopped and the next stimulus began after an interval of at least 10 min. Every threshold of 2000, 250 and 5 Hz was measured 3 or 4 times and the mean value of the intensities was expressed as the current perception threshold.

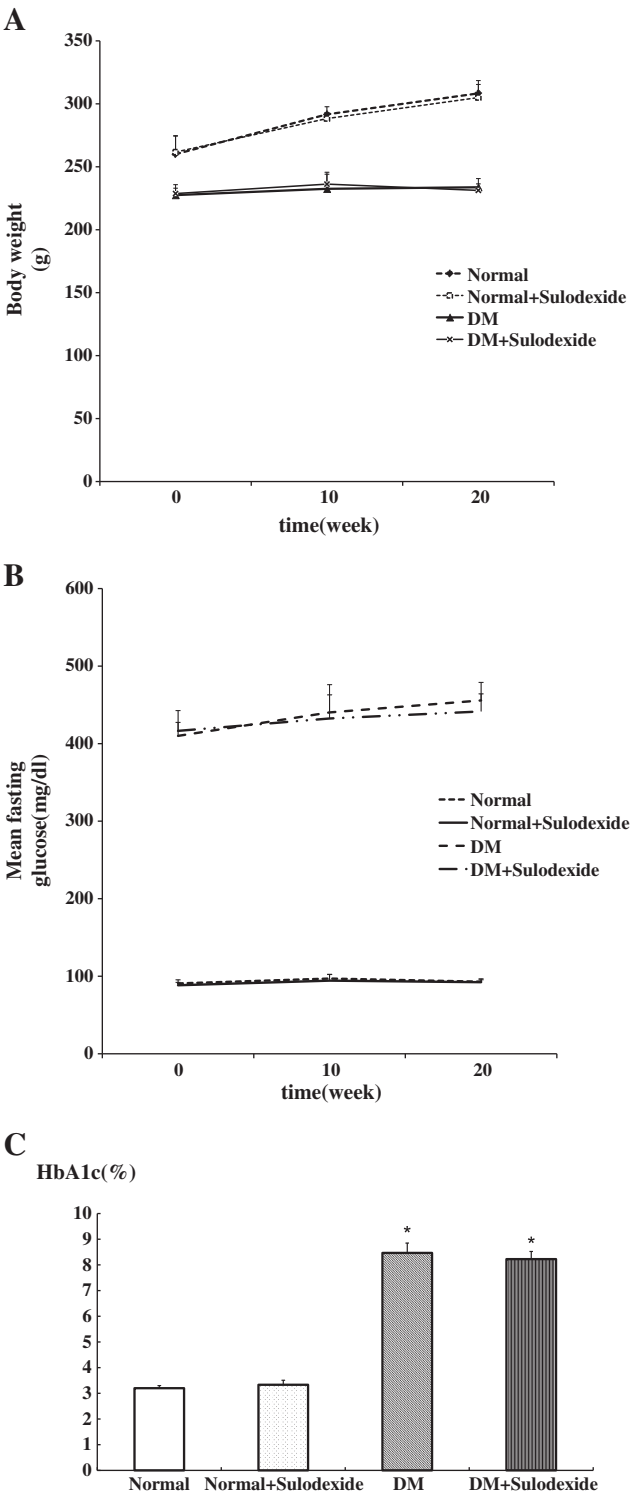

Fig. 1. (A) Body weight changes according to time. Body weight increases were not observed in the diabetes-induced rats, however normal glucose rats gained weight gradually during the experimental period. (B) Fasting blood glucose levels during the experimental period. Sulodexide administration did not result in the lowering of blood glucose in any of the groups. (C) HbA1c levels of the rats in the 20th week. HbA1c levels were also independent of sulodexide treatment in the normal and diabetic group. DM: Diabetes. N = 7–9 in each group. *: $P<0.05$ DM vs Normal. **: $P<0.05$ DM + Sulodexide vs DM.

2.5. Skin blood flow measurements

In week 20, dorsum tissue perfusion was evaluated by skin blood flow after treadmill running, as it was difficult to obtain significant